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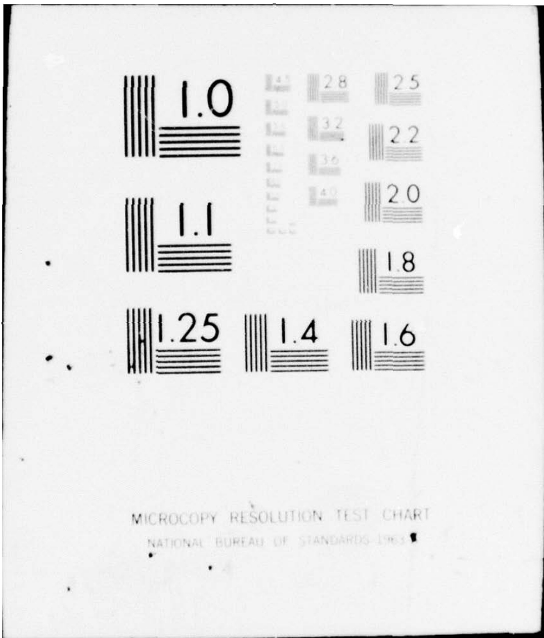
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A number of strains of Slamonella typhimurium were used to demonstrate endogenous mutagenesis by biologically-produced oxygen free radicals. Cells which are grown anaerobically, and therefore which possess low levels of superoxide dismutase are killed when exposed to oxygen in the presence of an oxidizable carbon source and puromycin. Aerobically-grown cells which contain high levels of superoxide dismutase survive identical treatment. A number of indicator strains were used to slow that exposure of anaerobically-grown cells

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to oxygen caused deletions, frameshift and apparent base substitution mutations. It also was shown that a recA⁻ mutant strain of S. typhimurium is more sensitive than is the wild type strain to oxygen exposure, but does not yield mutants, which shows that under the conditions described, direct damage to DNA is the cause of the enhanced mutation frequency. It is shown that, under appropriate conditions, oxygen can be used as a "clean" mutagenic agent for laboratory use.

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Identification of Types of Mutations Induced by Biologically - Produced Oxygen Free Radicals

Final Report

Richard O. Burns

December 30, 1976

U.S. Army Research Office

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INTRODUCTION

Oxygen radicals (O_2^-, OH·) are known to be produced by radiations of every sort. The reactivity of these radicals provide biological hazards and have been studied extensively by radiation biologists and radiation chemists. Recently, a new dimension to the toxicity of oxygen has become appreciated and is receiving a great deal of attention from biochemists. Biological reduction of oxygen usually involves the transfer of electron pairs. However, owing to the paramagnetic nature of the oxygen biradical (O_2) univalent pathways of oxygen reduction are favored over those involving electron pairs so that the products of univalent reduction are also produced (1). Only one of the products of univalent reduction was thought to be a consequent biological hazard; historically, H_2O_2 was always considered the culprit in undermining biological integrity. This view was not illogical, since the other two products (O_2^- and OH·) have extremely brief existence in aqueous environment. Also, extremely active enzymes, catalases and peroxidases, were known to rapidly remove H_2O_2 from biological systems and their presence in aerobic tissues were through to serve as the sole agents for protection against the reduced product of biological oxidation. However, the realization that many oxidases of the cell are capable of generating superoxide radicals (O_2^-), together with an appreciation of the vastly greater reactivity of oxygen free radicals, led to the discovery of additional enzymes which serve as principles of detoxification. We now know, primarily because of the work of Fridovich and his colleagues (2), that all aerobic cells produce superoxide dismutases which catalyze the reaction $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$ and that these serve as essential adjuncts to aerobic survival (3).

This report presents results of experiments employing Salmonella typhimurium to show that the sensitivity of superoxide dismutase deficient cells to O_2 is at least partly explained by "endogenous" mutagenesis and DNA damage. The exposure to oxygen of oxidatively-active cells which are deficient in superoxide dismutase mimics the action of ultraviolet irradiation; deletions, frameshift and point mutations are produced. Application of this principle in a general mutagenic procedure shows that, under appropriate conditions atmospheric oxygen (air) can be used as a "clean" mutagenic agent.

MATERIALS AND METHODS

Bacterial strains. Bacteria and phages used in this study are listed in Table I. All bacterial strains are derivatives of Salmonella typhimurium LT2.

Table I. Bacterial strains and phages

Strain or genetic marker	Characteristic	Source
LT-2	Wild type.	Standard strain carried in this lab since 1964.
leu500	single site, promoter-operator mutation in leu operon.	P. Margolin
leuD700	a deletion of the leuD gene.	P. Margolin
proAB	a deletion of the proAB genes.	P. Margolin (orin. M. Demerces)
DW292	Contains a lesion in recA gene (increased degradation of damaged DNA).	N. Kredich
leu500 suX	suX is a locus between cys & trp which can be deleted to restore prototrophy to leu500-bearing strain.	P. Margolin
leuA409	An amber mutation in the leuA gene.	P. Margolin (classified in this lab)
hisG46	hisG46 (base pair substitution)	B. Ames
TA100	hisG46 (base bair substitution) rfa, ΔuvrB + R (contains resistance transfer factor).	B. Ames
TA98	hisD3052 (frameshift), rfa, ΔurvB, R.	B. Ames
TA1537	hisC3076 (frameshift), rfa, ΔuvrB.	
TA1535	hisG46, rfa, ΔuvrB.	B. Ames
Coliphage T-7		D. Hall

Growth Conditions.

Cells were grown anaerobically in Davis-Mingioli medium (4), modified by omitting the citrate and reducing the concentration of glucose to 0.075%. Overnight nutrient broth (Difco) culture were used as a 0.5% inoculum for appropriately supplemented minimal medium which had been previously heated in a boiling water bath to expell dissolved oxygen and then rapidly cooled in an ice bath. The cultures were placed in a Torbal, Model AJ-2 anaerobic jar containing an atmosphere of 95% N_2, 5% CO_2 and incubated overnight at 37°. Cells were grown aerobically in the same medium at 37" in a New Brunswick Gyrotory shaker.

Exposure to hyperbaric O_2.

A stainless steel pressure vessel equipped with a pressure gauge and bleeding valve was used. Ten atmospheres of purified O_2 was applied to the vessel following a five minute purge of the vessel with O_2. Cells to be exposed to O_2 were usually placed in standard 100mm disposable plastic petri dishes.

Ampicillin selection.

Cultures were enriched for auxotrophic mutants by ampicillin selection. Cells in minimal medium supplemented according to the requirement of the parent strain were grown aerobically in a 37° water bath. The increase in optical density was monitored at 420nm and when in the midexponential growth phase twenty µg/ml of ampicillin was added. The optical density of the culture was monitored until it dropped to 50% of the initial value at which time the cells were centrifuged and washed with fresh medium. The surviving population was either scored for mutants by plating on appropriate medium or if the cells were to be recycled through another ampicillin selection procedure, they were grown overnight in appropriate medium and then carried through the above described procedure.

Superoxide dismutase assay.

Superoxide dismutase was measured according to the procedure of Gregory and Fridovich (5). Protein was measured by the method of Lowry, *et al.* (6).

RESULTS

Superoxide dismutase in Aneraobically- and aerobically-grown cells.

Preliminary experiments were performed to determine whether or not the levels of superoxide dismutase could be controlled by the conditions of growth of Salmonella typhimurium. The levels of superoxide dismutase was measured in crude extracts prepared from anaerobically- and aerobically-grown cells. The enzyme assay is based upon the ability of superoxide dismutase to prevent the reduction of cytochrome C by superoxide radicals generated during the oxidation of xanthine by xanthine oxidase (5). Table 1 shows the results of this analysis on two strains of S. typhimurium as well a on Escherichia coli B. This latter organism was included as a control because of a previous report that superoxide dismutase is oxygen-induced in this organism.

Table 2

Superoxide Dismutase in Aerobically and Anaerobically-Grown S. typhimurium

	Superoxide dismutase specific Activity (units/mg protein)	
	Anaerobic	Aerobic
LT2	6.7	12.0
leu500	5.0	11.0(30)*
E. coli B	2.5	8.7

*Specific activity of SOD following exposure of cells to 10 atm. of O_2 for 1.5 hours.

The results show that anaerobically-grown cells contain less superoxide dismutase than aerobically-grown cells. According to these results anaerobically-grown cells should display a greater oxygen toxicity than aerobically-grown cells.

Survival of anaerobically- and aerobically-grown cells to hyperbaric O_2.

S. typhimurium LT2 was grown anaerobically and aerobically in minimal medium. Puromycin (500μg/ml) was added to a portion of each culture immediately upon removal from their respective growth environment, each puromycin-culture mixture was made 0.5% with respect to glucose and placed in a hyperbaric chamber as described in Materials and Methods. Samples of each mixture were removed at various time intervals and the number of viable cells determined by colony counts on nutrient agar plates. Figure one shows the relative survival of the two populations of cells. It is clear from these results that, in the absence of protein synthesis, cells with the higher level of superoxide dismutase are more resistant to hyperbaric oxygen.

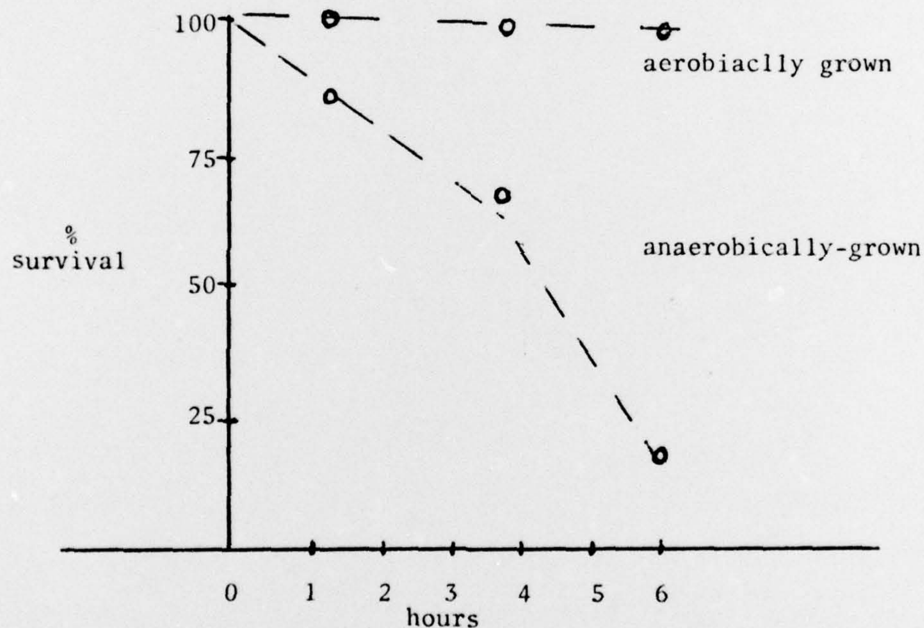

Figure 1. Survival of anaerobically- and aerobically-grown S. typhimurium. Cells were in minimal medium supplemented with 0.5% glucose and 500μg/ml puromycin. Ten atmospheres of O_2 was applied at 37°. Samples were removed at the intervals shown and plated on nutrient agar for colony counts.

The nature of the oxygen-induced damage in superoxide dismutase-deficient cells.

The results presented in Table 2 and Figure 1 suggest that oxygen toxicity in anaerobically-grown cells is because of low levels of superoxide dismutase and by inference, because of the failure to remove superoxide radicals. This view is supported by the data presented in Table 3 which shows the relative survival, under various conditions, of anaerobically and aerobically-grown cells during exposure to hyperbaric O_2. The results show: (1) aerobically-grown cells are resistant to hyperbaric oxygen under all conditions tested, (2) the effect of hyperbaric oxygen on anaerobically-grown cells is augmented by glucose which, being an oxidizable carbon source, enhances the production of superoxide radicals, (3) puromycin, by blocking protein synthesis (including superoxide dismutase) during exposure to hyperbaric oxygen, enhances the toxic effect of oxygen.

Table 3

Strain	Growth condition of cells prior to O_2 treatment	Additions during exposure to 10 atms. O_2 at 27C for 6 hours.			
		none	glucose	puromycin	puromycin + glucose
		% Survival			
LT2	aerobic	100	100	100	100
	anaerobic	70	56	50	32

The foregoing results strongly suggest that the superoxide radical, or a product of superoxide radicals, is the toxic agent responsible for killing anaerobically-grown cells. One of the most likely targets for damage is the DNA of the cell. To test this possibility a derivative of S. typhimurium which bears a mutation in the recA gene was grown aerobically and anaerobically and the relative sensitivity to hyperbaric oxygen tested. The recA gene produces a protein which is involved in general genetic recombination (7). Strains lacking this function are unable to effect post-replication repair of damaged DNA, and more significantly, for the

purposes of the present analysis, extensively degrade their DNA following damage by such agents as ultraviolet light, x-irradiation and alkylation (8,9,10). If hyperbaric O_2 causes damage to the DNA of anaerobically-grown cells then the recA⁻ strain should be more sensitive to this treatment than is the wild type strain. Table 4 presents a compilation of the results of several experiments in which the relative sensitivities of three strains of *S. typhimurium*, including the recA⁻ strain are compared.

Table 4

Relative survival of three strains of *S. typhimurium* to hyperbaric oxygen.

Strain	Growth Condition	exposure to: O_2	exposure to: O_2 + puromycin
LT2 (wild type)	anaerobic	56 (5)*	32 (5)
	aerobic	100 (2)	100 (2)
Leu500	anaerobic	37 (13)	21 (13)
	aerobic	83 (2)	100 (2)
DW292 (recA⁻)	anaerobic	9 (9)	4 (6)
	aerobic	80 (9)	100 (6)

*( ) = number of experiments.

The cells were grown and exposed to oxygen as previously described. All cell suspensions contained 0.5% glucose.

The results in Table 3 demonstrate the reproducibility of the relative toxic effect of O_2. Strain DW292(recA⁻) is particularly sensitive to hyperbaric oxygen; this observation strongly supports the notion that DNA is the primary target for the lethal effect of O_2.

Mutagenic action of hyperbaric O_2.

A number of tester strains were used to determine whether or not the putative damage to DNA wrought by hyperbaric oxygen in cells

undergoing oxidative metabolism in the presence of low levels of superoxide dismutase is reflected in an enhanced mutation frequency. Strains specifically designed for detection of deletion mutations as well as strains to detect point mutations were used.

DELETION MUTATIONS. Two tester strains were employed to determine whether or not the frequency of deletion-containing strains was increased upon exposure to hyperbaric oxygen.

The leuD - suQ system (11). Isopropylmalate isomerase, an enzyme which is specifically involved in leucine biosynthesis, is composed of two different polypeptide chains, one specified by the leuC gene and the other by the leuD gene. Deletion mutations lacking the leuD gene can be suppressed by mutation of the suQ gene. Many suQ suppressors of leuD are deletions which extend into the neighboring pro region and are recognized as such because of an attendant proline auxotrophy. The frequency of Leu^+ prototrophs following treatment of the leuD strain as compared with the frequency from untreated cells is given in the following Table.

Table 5

Increased Frequency of suQ Mutations

Treatment	Mutations/10^9 cells	Types of Mutations	
		Leu^+ Pro^+	Leu^+ Pro^-
Control	1.2	100%	0%
Oxygen	6.0	80%	20%
Oxygen & puromycin	13.6	90%	10%

Mutation frequency of anaerobically grown cells was compared before and after treatment with 10 atm. O_2 for 4 hours. The Pro^- colonies were scored by replica plating from minimal-proline plates to minimal plates. The tester system described above strongly suggests that hyperbaric oxygen increases mutation frequency and that deletion mutations represent a significant portion of the mutant population.

leu500 suX system (12). The auxotrophy imparted by the leu500 lesion is suppressible by mutations in the suX locus. The suX locus is flanked by the cysB and trp loci. Many suX mutations are deletions which extend into the cysB region, or the trp region or both the cysB and trp regions; these types of deletions result in leucine prototrophy and cysteine, tryptophan or cysteine-tryptophan auxotrophy. The following Table presents the results of an experiment which is similar to the one described above.

Table 6

Increased Frequency of suX Mutations

Treatment	Mutations/10^7 cells	Types of Mutations			
		cys^-	trp^-	cys^--trp^-	prototrophs
Control	9.9	1.6%	1.4%	1.6%	95.4%
Oxygen	14	0.68%	5.14%	0.68%	93%
Oxygen & puromycin	37	1.1%	1.1%	1.4%	95%

These results show that in spite of a high frequency of spontaneous suX mutation hyperbaric oxygen enhances this frequency.

These results with the leuD -suQ and leu500-suX system raise the possibility that the increase in mutation frequency is but an apparent one with the pertinent mutations being selected by the hyperbaric oxygen rather than being caused by it. The method of computation of the relative mutation frequencies does not rule this out since these are based upon the number of surviving organisms rather than upon the input number of cells. In order to rule out selection as the cause of the increased mutation frequency the relative survival of a well characterized suX strain was compared with that of leu500. The results of this experiment are given in the following Table.

Table 7

Comparative survival of Leu500 and Leu500 suX strains to hyperbaric O_2.

Exposed to:	% survival	
	leu500	leu500 suX
O_2	48	31
O_2 + puromycin	43	44

Cells were grown and exposed to O_2 for 4 hrs as previously described.

SINGLE SITE MUTATIONS. The foregoing tester strains conclusively illustrate that hyperbaric oxygen causes multisite (deletion) mutations. The question remains as to whether or not single site mutations are also caused by hyperbaric oxygen.

In order to test this possibility strains developed by Ames and his coworkers were used (13). The experimental procedure was similar to that employed above. The various tester strains were grown anaerobically, and then exposed to 10 atm. 0_2 for 4 hours in the presence and in the absence of puromycin.

Table 8

Reversion to Histidine Prototrophy of His⁻ Tester Strains

Strain	Mutations/10^7 cells		
	Control	$+0_2$	$+0_2$ + puromycin
TA 1537	7.9	95	410
TA 98	1.6	73	88
TA 100	77	640	390
TA 1535	21	79	230

Mutation frequency is computed as the number of histidine prototrophs per total colony forming unit. All suspensions contained 0.5% glucose.

Strains TA 1537 and TA 98 are indicators of frameshift mutagenesis and strains TA 100 and TA 1535 each contain the same base pair substitution in the hisG gene. The foregoing results suggest that hyperbaric oxygen causes both frameshift mutations as well as base pair substitutions.

Oxygen as a "clean" mutagenic agent for laboratory applications.

Many of the mutagens (viz. ethylmethane sufonate, nitrosoquanidine) used in microbiology laboratories, also are powerful carcinogens and therefore are extremely hazardous. The foregoing results suggest that in those organisms where the levels of superoxide dismutase can be altered by growth conditions that exposure to oxygen, in the absence of protein synthesis, leads to enhanced mutation frequencies. In order to make the application of this principle as simple as

possible preliminary tests were performed to estimate the survival of anaerobically-grown cells, treated as described for the hyperbaric experiments described above, but exposed to air by shaking in a standard gyrotory water bath at 37°. These experiments yielded surprising results. It was found that the survival patterns of anaerobically- and aerobically-grown cells when exposed to atmospheric O_2 (air) mimiced the patterns in the hyperbaric experiments, i.e., hyperbaric O_2 is not required to demonstrate the toxic effect of O_2. In order to show that the toxic effect of atmospheric O_2 was qualitatively the same as the effect of hyperbaric O_2 the relative survival of anaerobically-grown wild type _S. typhimurium_ was compared with the _recA⁻_ strain: Each strain was grown anaerobically and then incubated at 37° with shaking in the presence of the standard concentration of puromycin and glucose. The survival after 4 hours exposure was 32% for wild type and 13% for the _recA⁻_ strain.

The feasability of oxygen mutagenesis was tested by randomly selecting amino acid auxotrophs from populations of cells which were grown anaerobically and aerobically. Anaerobically-grown cells were incubated with and without puromycin at 37° in a gyrotory water bath for four hrs., washed with saline and then grown overnight in nutrient broth to allow expression of mutants. These cells were washed with saline, placed into minimal medium and carried through on ampicillin enrichment step (ampicillin kills dividing cells, therefore mutants unable to grow in minimal medium will survive). these cells were then washed, resuspended in nutrient broth and grown overnight, after which a second ampicillin enrichment procedure was performed. The cells were washed and again grown overnight in nutrient broth. The cells from this culture were diluted and plated for single colonies on nutrient agar. This entire procedure was also conducted with cells which were grown aerobically and incubated with puromycin in parallel with the anaerobically-grown cells. The single colonies which appeared on the nutrient agar plates were replica-plated onto minimal medium and the frequency of auxotrophs recorded. Those colonies which did not grow on minimal medium were purified and classified according to auxotrophic requirement. Table 9 shows the frequency of auxotrophs in the three samples. Table 10 shows the array of auxotrophic requirements among the mutant strains.

These results show that anaerobically-grown cells yield a wide assortment of mutants following exposure to oxygen in the absence of protein synthesis. The types of mutations appear to be varied and the numbers in each category cannot be explained solely on the basis of clonal amplification during growth in the nutrient broth prior to plating. This last contention is borne out by the observation that whereas some members of a class are able to revert to prototrophy others are not.

Table 9

Mutagenesis of S. typhimurium LT2

Growth conditiond prior to exposure to O_2	Exposure of O_2 in presence of:	No. mutants/total no. Colonies
Anaerobic	puromycin	116/627 = 18.5%
Anaerobic	none	30/537 - 5.6%
Aerobic	puromycin	5/480 = 1.0%

Table 10

Characterization of O_2 induced mutants of S. typhimurium

Source	Requirement										
	cys	his	ileu	ileu/val	leu	met	phe	pro	ser	tyr	bradytroph†
Anaerobic + O_2 + puromycin	0	2(1*)	2	0	36(16)	10(4)	1	5(2)	15(6)	2(1)	45
Anaerobic + O_2	1(1)	1(1)	0	2	15(4)	1	0	0	7(1)	1(1)	2

*The numbers in paraenthesis represent the members of each group which revert to prototrophy.
†Bradytrophs are "leaky" mutants, i.e., grow slow on minimal medium.

The results presented in Table 10 reveal another significant feature as regards oxygen mutagenesis, namely, that no double mutants were found. This observation indicates that oxygen mutagenesis potentially is a valuable laboratory tool, circumventing a major problem encountered with many mutagens, namely, multiple mutations.

The lack of oxygen-induced mutagenesis in a $recA^-$ strain.

Strains of bacteria which lack the recA functions although extraordinarily sensitive to agents which damage DNA, do not yield mutant strains when exposed to potentially mutagenic agents such as ultraviolet light (14). In order to verify the interpretation of the results presented in this report, and to demonstrate that oxygen induced mutagenesis involves direct damage to DNA, the general mutagenesis procedure described above was repeated with strain DW292 ($recA^-$). No mutants were found.

DISCUSSION

The foregoing results show that the lethal effect of oxygen on cells which contain low levels of superoxide dismutase is at least partially explained in terms of damaged DNA. This does not mean, however, that the damage which apparently is induced by oxygen free radicals is exclusively directed toward the DNA of the cell. Another candidate for damage would be the cell membrane in as much as free radicals would be expected to react with unsaturated fatty acids. Although this point was not addressed directly in this project, preliminary results in the form of electron micrographs suggest that membrane damage could be occurring. Micrographs of oxygen-exposed cells show a large number aberrant forms with predominating folded, cigar-shaped bodies.

The data included in this report do not permit identification of the active mutagenic primicple, however, if the active agent is not the superoxide radical it most certainly is derived from it. For example, the hydroxyl radical (OH·) could be involved in the observed mutagenesis; this extraordinarily reactive oxidant would be formed by a reaction proposed by Haber and Weiss in 1934, ($O_2^- + H_2O_2 \rightarrow OH^- + OH\cdot + O_2$). Regardless of the precise nature of the mutagenic agent it appears clear that superoxide dismutase protects cells from the toxic effect of oxygen and in doing so prevents damage to DNA.

The primary goal of this research was to determine whether or not the toxic effect of oxygen in superoxide dismutase-deficient cells would be expressed in an enhanced mutation frequency, and if so, to define the types of mutations. Employing appropriate tester strains of *S. typhimurium* it was shown that the frequency of deletions, frameshift, and base substitutions is enhanced by exposing oxidatively active, superoxide dismutase-deficient cells to oxygen in the absence of protein synthesis. It appears, therefore, that the spectrum of mutations, under these conditions, mimics the effect of ultraviolet and higher energy irradiation.

The results of the experiments which were designed to show the general mutagenic effect of oxygen revealed no bias with respect to susceptible regions of the chromosome. Although it appears from the data presented in Table 10 that a bias does exist for Leu^- mutants, this is explained by the ampicillin selection procedure. The relatively abundant amino acid in the protein of *S. typhimurium* is L-leucine. The survival of the various auxotrophs during the ampicillin enrichment procedure depends upon the inability of these to grow; only actively dividing cells are killed by ampicillin. Owing to the high requirement which cells have for L-leucine this amino acid will be removed most efficiently from the endogenous carryover nutrients; i.e., the period of growth following the transfer of cells from nutrient broth to minimal medium and preceding the addition of ampicillin will permit most efficient removal of L-leucine and consequent enhanced survival of leucine auxotrophs.

An additional revelation is that the general mutagenesis resulted in the apparent lack of double or multiple mutations among the organisms surviving exposure to oxygen. Although this observation may reflect the relatively gentle nature of "endogenous" mutagenesis, it may also reflect some undefined property of this mutagenic process.

In summary, this research serves to describe the role played by the reduced products of oxygen in "endogenous" mutagenesis and affirms the role of oxidative metabolism in augmenting the subtle background which serves, in part, as the active agent for promoting so-called spontaneous mutants and demonstrates the role of superoxide dismutase in dampening the frequency of these mutations.

The work described in this is presently being prepared for publication and will be submitted to the *Journal of Bacteriology*; reprints will be submitted when available.

BIBLIOGRAPHY

1. Taube, H. 1965. Oxygen: Chemistry, Structure and Excited States. Boston, Little, Brown.

2. McCord, J. M., & I. Fridovich. 1968. J. Biol. Chem. 243, 5753.

3. McCord, J. M., B. B. Keele & I. Fridovich. 1971. Proc. Nat. Acad. Sci. USA. 68: 1024.

4. Davis, B. D. and E. S. Mingioli. 1950. J. Bacteriol. 60: 17-28.

5. Gregory, E. M. & I. Fridovich. 1973. J. Bacteriol. 114: 543.

6. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. J. Biol. Chem. 193: 265.

7. McEntee, K., J. E. Hesse & W. Epstein. 1976. Proc. Nat. Acad. Sci. USA. 73: 3979.

8. Clark, A. J. & A. D. Margulies. 1965. Proc. Nat. Acad. Sci. USA. 53: 451.

9. Howard-Flanders, P. & L. Theriot. 1966. Genetics 53: 1137.

10. Jenkins, S. T. & P. M. Bennet. 1976. J. Bacteriol. 125: 1214.

11. Kemper, J. & P. Margolin 1969. Genetics 63: 263.

12. Mukai, F. H. & P. Margolin. 1963. Proc. Nat. Acad. Sci. USA 50: 140.

13. Ames, B. N., J. McCann & E. Yamasaki. 1975. Mutation Research 31: 347.

14. Brooks, D. & A. J. Clark 1967. J. Virol. 1: 283.